INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2004/014704

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C12N15/09, C12P21/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C12N15/09, C12P21/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
BIOSIS/WPI(DIALOG), MEDLINE(STN), JSTPlus/JST7580(JOIS),
SwissProt/PIR/GeneSeq, GenBank/EMBL/DDBJ/GeneSeq

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X/Y	Kramer R.A. et al., Identification of essential acidic residues of outer membrane protease OmpT supports a novel active site, FEBS Lett, 2001, Vol.505, No.3, pages 426 to 430	12,15,27, 31-35/13-14, 16-17,21-22
Y	OKUNO K. et al., Substrate specificity at the P1' site of Escherichia coli OmpT under denaturing conditions, Biosci Biotechnol Biochem, 2002, Vol.66, No.1, pages 127 to 134	13-14,16-17, 21-22
A	Dekker N. et al., Substrate specificity of the integral membrane protease OmpT determined by spatially addressed peptide libraries, Biochemistry, 2001, Vol.40, No.6, pages 1694 to 1701	1-35

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 December, 2004 (13.12.04)	28 December, 2004 (28.12.04)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (January 2004)

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International application No.
PCT/JP2004/014704

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OKUNO K. et al., An analysis of target preferences of Escherichia coli outer-membrane endoprotease OmpT for use in therapeutic peptide production: efficient cleavage of substrates with basic amino acids at the P4 and P6 positions, Biotechnol. Appl. Biochem., 2002, Vol.36(Pt 2), pages 77 to 84	1-35
P,X	OKUNO K. et al., Utilization of Escherichia coli outer-membrane endoprotease OmpT variants as processing enzymes for production of peptides from designer fusion proteins, Appl. Environ. Microbiol., 2004 Jan, Vol.70, No.1, pages 76 to 86	1-35

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(See extra sheet.)

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

Continuation of Box No.III of continuation of first sheet(2)

(1) The inventions according to claims 1 to 7 and the parts relating to claims 1 to 7 in claims 9 to 11 and 31 to 35 relate to a method of cleaving a polypeptide at a desired cleavage site by using OmpT protease in the case where the amino acid at the P1-position of the desired cleavage site of the polypeptide is arginine or lysine, the amino acid at the P1'-position thereof is one other than aspartic acid, glutamic acid or proline, and one basic amino acid or two or three consecutive basic amino acids are located at an arbitrary part in the amino acid sequence of from P10- to P3-positions or from P3'- to P5'-positions (provided that in the case of having one basic amino acid, it is located at a position other than the P6- or P4-position).

(2) The inventions according to claims 8 and 23 and the parts relating to claims 8 and 23 in claims 9 to 11 and 24 to 35 relate to a method of cleaving a polypeptide or a fused protein at a desired cleavage site by using OmpT protease wherein, in the case where the polypeptide or the fused protein has a site not desired to be cleaved with OmpT protease, an acidic amino acid is located at the P3-position of the corresponding site.

(3) The inventions according to claims 12 and 15 and the parts relating to claims 12 and 15 in claims 18 to 22 and 25 to 35 relate to a method of cleaving a polypeptide at a desired cleavage site by using an OmpT protease mutant in which the amino acid at the 97th position from the N-end is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

(4) The inventions according to claims 13 and 16 and the parts relating to claims 13 and 16 in claims 18 to 22 and 25 to 35 relate to a method of cleaving a polypeptide at a desired cleavage site by using an OmpT protease mutant in which the amino acid at the 97th position from the N-end is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, in the case where the amino acid at the P1-position of the desired cleavage site of the polypeptide is arginine or lysine and the amino acid at the P1'-position is one other than arginine or lysine.

(5) The inventions according to claims 14 and 17 and the parts relating to claims 14 and 17 in claims 18 to 22 and 25 to 35 relate to a method of cleaving a polypeptide at a desired cleavage site by using an OmpT protease mutant in which the amino acid at the 97th position from the N-end is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, in the case where the amino acid at the P1-position of the desired cleavage site of the polypeptide is arginine or lysine, the amino acid at the P1'-position is one other than arginine or lysine, and one, two or three basic amino acids are located at an arbitrary part in the amino acid sequence of from P10- to P3-positions or from P3'- to P5'-positions.

(continued to next page)

However, there has been publicly known a method of cleaving a polypeptide at a desired cleavage site by using OmpT protease, in the case where the amino acid at the P1-position of the desired cleavage site is arginine or lysine, the amino acid at the P1'-position is one other than arginine or lysine, and one, two or three basic amino acids are located at an arbitrary part in the amino acid sequence of from P10- to P3-positions or from P3'- to P5'-positions, as reported in Biosci. Biotechnol. Biochem., 2002, Vol.66, No.1, pp.127-134. Also, there has been publicly known a method of cleaving a polypeptide at a desired cleavage site by using a mutant of mpT protease having a mutation at the amino acid at the 97th position from the N-end, as reported in FEBS Letters, 2001, Vol.505, pp.426-430. Thus, none of the matters common to any of the above items (1) to (5) can be considered as a special technical feature.

Such being the case, the inventions as claimed in the claims of the present case have five groups of inventions.